Supplemental Information

Two microRNA regulatory circuits set start and end times for dendritic arborization of a nociceptive neuron

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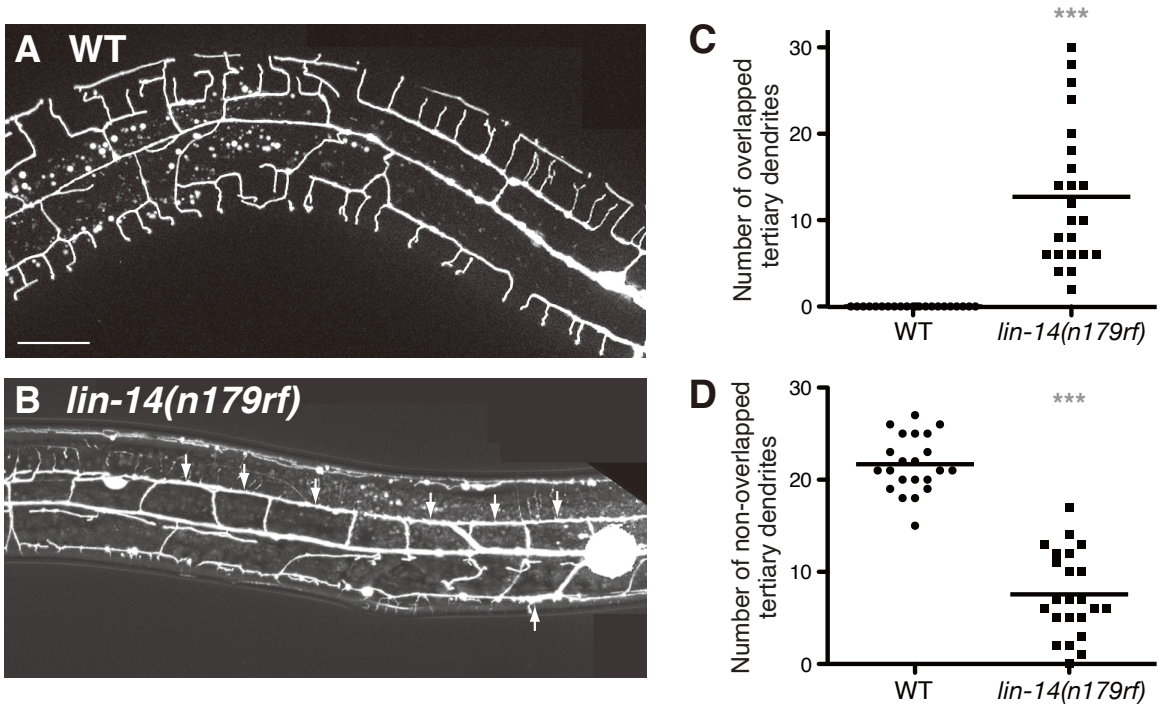

Figure S1. *lin-14(n179rf)* mutations caused excessive PVD dendrite outgrowth, related to Figure 2. (**A, B**) Images of PVD dendrites in wild type (**A**) and *lin-14(n179rf)* mutants (**B**). Dorsal is up; anterior is to the left. Scale bar, 20 μm. *lin-14(n179rf)* mutants displayed excessive tertiary dendrites. Arrows point to contacts between neighboring tertiary dendrites, which are absent in wild-type animals. (**C**) Quantification of the number of overlapped tertiary dendrites in wild type versus *lin-14(n179rf)* mutants. (**D**) Quantification of the number of non-overlapped tertiary dendrites in wild type versus *lin-14(n179rf)* mutants. Each dot represents data from a single animal. ***$p < 0.001$ by a Student's *t*-test.

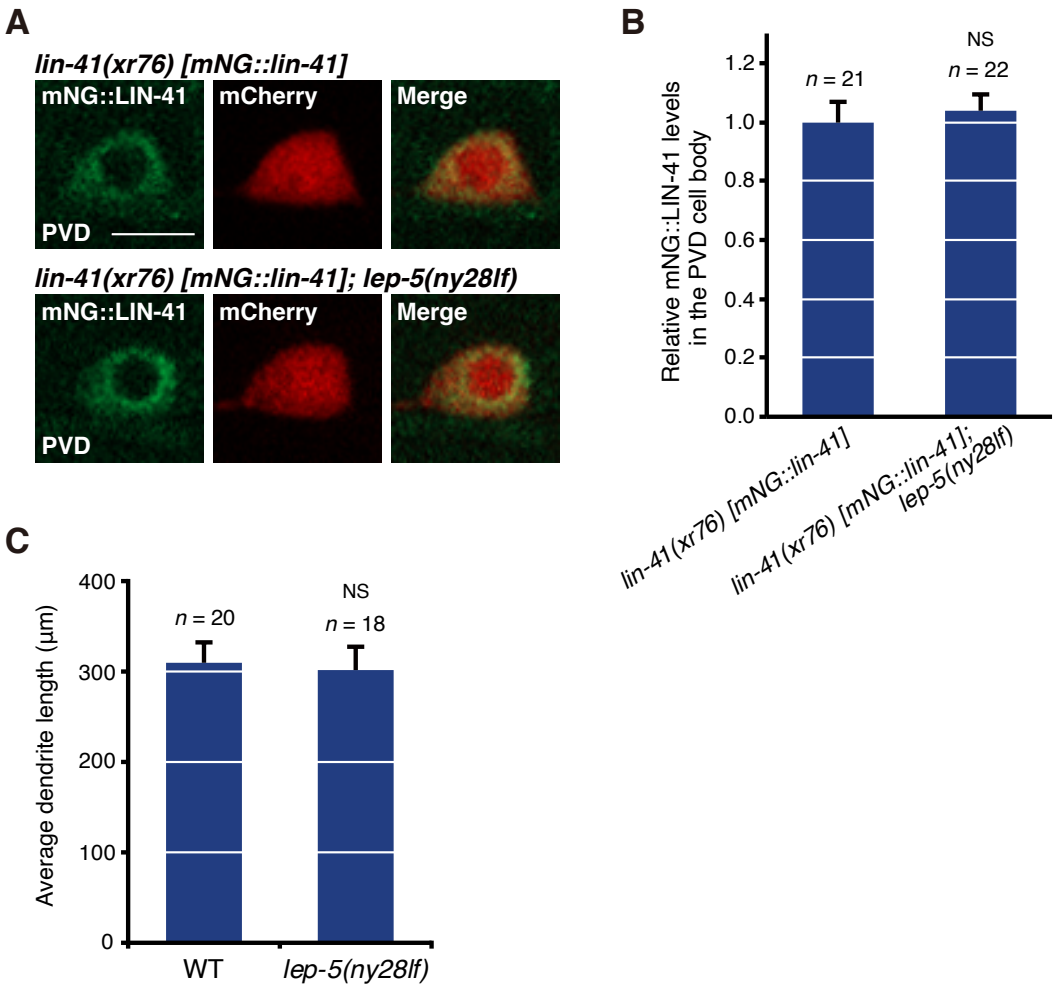

Figure S2. *lep-5* does not regulate dendrite growth ability, related to Figure 3. (**A**) Representative images of the expression of endogenous LIN-41 proteins in PVD neurons in wild type and *lep-5(ny28lf)* mutants at the early L3 stage. The *Pser-2::mCherry* reporter was used to label PVD neurons. Scale bar, 5 μm. (**B**) Quantification of endogenous LIN-41 proteins based on the fluorescence intensity in the PVD cell body in wild type and *lep-5(ny28lf)* mutants. (**C**) Average dendrite length regrown in wild type and *lep-5(ny28lf)* mutants 24 hours following dendritomy of the primary dendrite at the young adult stage. Error bars, SEM. NS, not significant by a Student's *t*-test.

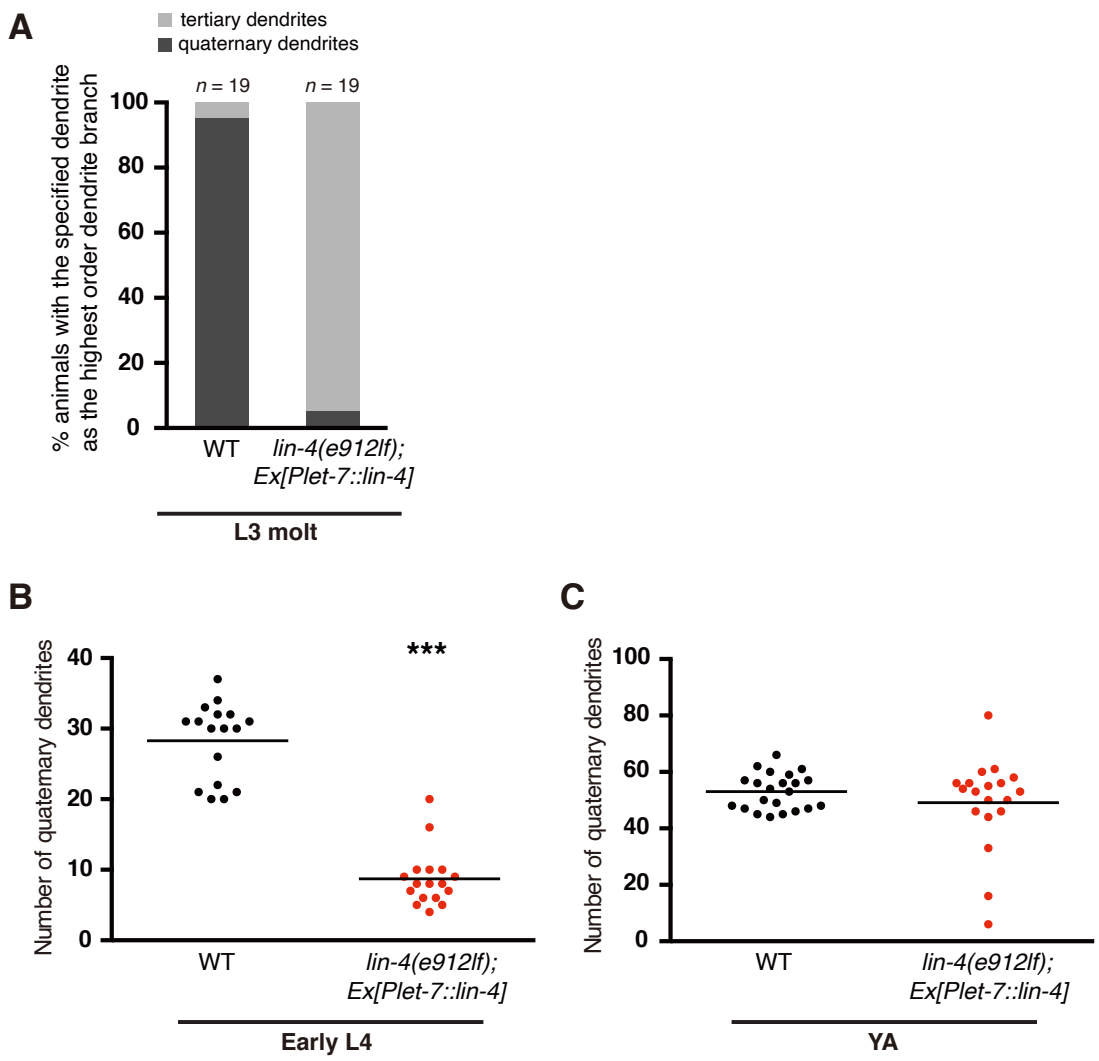

Figure S3. Delayed dendrite arborization by the *lin-4* to *let-7* promoter replacement, related to Figure 4. (**A**) Re-expression of the *lin-4* microRNA in *lin-4(e912lf)* mutants using a late-onset *let-7* promoter postponed growth of the quaternary dendrites. (**B, C**) Quantification of the number of quaternary branches per 250 μm in the anterior direction from the PVD cell body at the early L4 stage (**B**) or the young adult stage (**C**) in wild type and *lin-4(e912lf)* mutants carrying the *Plet-7::lin-4* transgene. ***p < 0.001 by a Student's *t*-test.

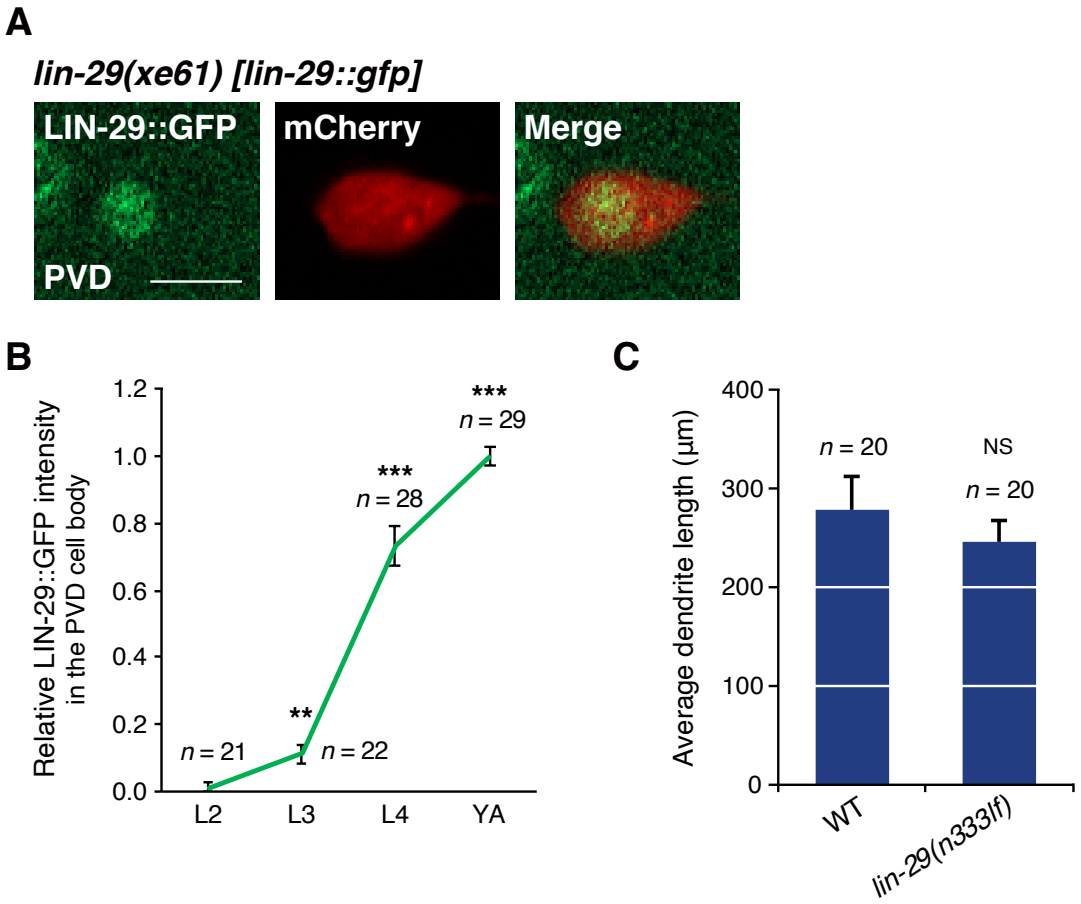

Figure S4. *lin-29* is not involved in regulating dendrite growth ability despite its expression in PVD neurons. (**A**) Representative images of the expression of endogenous LIN-29 proteins in PVD neurons in wild type at the young adult stage. The *Pser-2::mCherry* reporter was used to label PVD neurons. Scale bar, 5 µm. (**B**) The endogenous LIN-29 protein is temporally regulated in PVD neurons. Average fluorescent intensity of LIN-29::GFP proteins at four different developmental stages. Error bars, SEM. **p < 0.01 and ***p < 0.001, relative to the preceding stage, by a Student's *t*-test. (**C**) Average dendrite length regrown in wild type and *lin-29(n333lf)* mutants 24 hours following dendritomy of the primary dendrite at the young adult stage. Error bars, SEM. NS, not significant by a Student's *t*-test.

Table S1. *C. elegans* strains used in this study

Strains	Mutations	Integrated transgenes	Extrachromosomal transgenes
XN1588		*xrIs21[Plin-4::GFP] X*	*xrEx537[PF49H12.4::mCherry]*
XN1540			*xrEx425[Plin-14::GFP]; xrEx537[PF49H12.4::mCherry]*
XN1587			*xrEx533[Plet-7::GFP]; xrEx537[PF49H12.4::mCherry]*
XN1586			*xrEx117[Plin-41::GFP]; xrEx537[PF49H12.4::mCherry]*
XN1427		*xrIs21[Plin-4::GFP] X*	*xrEx514[Plin-14::mCherry]*
XN1422			*xrEx303[Plet-7::GFP]; xrEx518[Plin-41::mCherry]*
XN1808	*lin-4(e912) II*	*xrIs37[PF49H12.4::GFP] IV*	
XN1564	*lin-14(n355) X*	*xrIs37[PF49H12.4::GFP] IV*	
XN1555	*lin-14(n179) X*	*xrIs37[PF49H12.4::GFP] IV*	
XN2033	*lin-4(e912) II; lin-14(n179) X*	*wyIs581[Pser-2::mCherry]*	
XN2044		*xrIs37[PF49H12.4::GFP] IV*	*xrEx751[Pser-2::lin-14]*
XN2059	*lin-14(n179) X*	*xrIs37[PF49H12.4::GFP] IV*	*xrEx751[Pser-2::lin-14]*
XN1467		*xrIs37[PF49H12.4::GFP] IV*	
XN1825	*let-7(n2853) X*	*xrIs37[PF49H12.4::GFP] IV*	
XN1547	*lin-41(n2914)/unc-29(e1072) lin-11(n1381) I*	*xrIs37[PF49H12.4::GFP] IV*	
XN2072	*lin-41(n2914)/unc-29(e1072) lin-11(n1281) I; let-7(n2853) X*	*xrIs37[PF49H12.4::GFP] IV*	
XN2818		*wgIs535[lin-28 fosmid::GFP]*	*xrEx1157[Pser-2::mCherry]*
XN2540	*lin-41(xr76) [mNG::lin-41] I*		*xrEx997[Pser-2::mCherry]*
XN2637	*lin-28(n719) lin-41(xr76) I*		*xrEx1065[Pser-2::mCherry]*
XN2690	*lin-28(n719) I*		*xrEx1097[Pser-2::GFP]*
XN2691	*lin-28(n719) I*		*xrEx1098[Pser-2::lin-41; Pser-2::GFP]*
XN2693	*lin-28(n719) I*		*xrEx1100[Pser-2::lin-41; Pser-2::GFP]*
XN2054	*lin-4(e912) II*	*xrIs37[PF49H12.4::GFP] IV*	*xrEx744[Plet-7::lin-4]*
XN2209	*lin-4(xr70) [Plet-7::lin-4] II*	*xrIs37[PF49H12.4::GFP] IV*	
XN2210	*lin-4(xr71) [Plet-7::lin-4] II*	*xrIs37[PF49H12.4::GFP] IV*	
XN2204	*let-7(xr67) [Plin-4::let-7] X*	*xrIs37[PF49H12.4::GFP] IV*	
XN2205	*let-7(xr68) [Plin-4::let-7] X*	*xrIs37[PF49H12.4::GFP] IV*	
XN2670	*lin-41(xr76) I; lep-5(ny28) X*		*xrEx997[Pser-2::mCherry]*
XN2460	*lep-5(ny28) X*	*xrIs37[PF49H12.4::GFP] IV*	
TV18817	*dma-1(wy996) [dma-1::YFP] I*		
XN2233	*dma-1(wy996) I; lin-14(n179) X*		
XN1657	*dma-1(xr50) I*	*xrIs37[PF49H12.4::GFP] IV*	
XN2365	*dma-1(xr50) I; lin-14(n179) X*	*xrIs37[PF49H12.4::GFP] IV*	
XN2241	*dma-1(wy996) lin-41(n2914)/unc-29(e1072) lin-11(n1281) I*		
XN2725	*lin-29(xe61) [lin-29 a/b::gfp::3x flag] II*		*xrEx1126[Pser-2::mCherry]*
XN2266	*lin-29(n333) II*	*xrIs37[PF49H12.4::GFP] IV*	
XN1771	*lin-41(ma104) I*	*xrIs37[PF49H12.4::GFP] IV*	
XN2807		*xrIs37[PF49H12.4::GFP] IV*	*xrEx1152[Pser-2::dma-1]*
XN2810		*xrIs37[PF49H12.4::GFP] IV*	*xrEx1155[Pser-2::dma-1]*
XN2812	*lin-41(ma104) I*	*xrIs37[PF49H12.4::GFP] IV*	*xrEx1152[Pser-2::dma-1]*
XN2813	*lin-41(ma104) I*	*xrIs37[PF49H12.4::GFP] IV*	*xrEx1155[Pser-2::dma-1]*

Table S2. Plasmids used in this study

Plasmids
Plin-4::GFP
Plin-14::GFP
Plet-7::GFP
Plin-41::GFP
PF49H12.4::mCherry
Plin-14::mCherry
Plin-41::mCherry
PF49H12.4::GFP
Pser-2::lin-14
Pser-2::mCherry
Pser-2::GFP
Pser-2::lin-41
Plet-7::lin-4
PDD240::Plin-4 left::Plet-7::Plin-4 right repair template
Plin-4 sgRNA11 cas9
Plin-4 sgRNA12 cas9
PDD240::Plet-7 left::Plin-4::Plet-7 right repair template
Plet-7 sgRNA6 cas9
Plet-7 sgRNA8 cas9
Pser-2::dma-1